FLUX AND METABOLITE FLEXIBILITY IN *ESCHERICHIA COLI* AT SECONDS TIME SCALE IN RESPONSE TO RAPID SHIFTS OF SUBSTRATE EXCESS

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The construction of dynamic metabolic models, with the aim to understand and subsequently re-design the metabolism of organism of interest, requires accurate experimental data on metabolite concentrations and fluxes. Recently we presented a method to resolve the rapid (seconds time scale) dynamics of the growth rate of *Escherichia coli* in a pulse experiment [1], based on the dynamic liquid-phase mass balance for oxygen and the pseudo-steady-state ATP balance. When *E. coli* cells cultured in a glucose-limited chemostat at a dilution rate D=0.1 h^{-1} (C_S~10 mg/l) were suddenly exposed to glucose excess (C_S~500 mg/l), a 3-4 fold increase in the growth rate within 40-60 s was observed. This observation, which was supported with independent measurements of intracellular metabolites, implies a 3-4 fold increase in the rate of protein synthesis by the ribosomes within tens of seconds. Surprisingly, the observed increase in growth rate, and hence protein synthesis rate, was not matched by a significant increase in intracellular free amino acid concentrations. This ability of *E. coli* cells to rapidly change in the growth rate upon a sudden relief of substrate limitation should be taken into consideration in the construction of those dynamic models.

In this study, perturbation experiments were carried out with glucose and two different gluconeogenic substrates (pyruvate and succinate) on an aerobic glucose-limited chemostat of *E. coli* to further investigate this phenomenon. We show that growth rate increases instantaneously to the same extent after each substrate perturbation. No by-products were secreted after any of the added glycolytic (glucose) and gluconeogenic (pyruvate, succinate) substrate pulses. After each perturbation a pseudo steady state in flux and metabolites was established in about 30-40 s and a high oxygen uptake capacity of the cells was observed.

Furthermore, the growth rate increased within 40 s from its steady-state value of 0.13 h^{-1} to 0.3 h^{-1}. This is less than 50 % of the maximum value, which could indicate a capacity limit in e.g. the ribosomes. The *in vivo* dynamic responses showed massive reorganization and flexibility (1/100 to 14 fold change) of metabolic fluxes, including flux reversal of reactions, matching with large changes in the concentrations of intracellular metabolites. This resulted in dynamic shifts in the reaction quotients of pseudo/near equilibrium reactions. The *in vivo* kinetics of the near equilibrium reactions could be described well by the recently proposed, thermodynamically inspired “Q-linear kinetics” [2].

References

[1] Taymaz-Nikerel H et al., 2011. Metabolic Engineering, 13(3): 307-318.
[2] Canelas AB et al., 2011. Metabolic Engineering, 13(3): 294-306.